Materials Science & Engineering C 90 (2018) 420–432

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Potential of silk sericin based nanofibrous mats for wound dressing applications

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ARTICLE INFO

Keywords:
Silk
Sericin
Nanofibers
Antioxidant
Chronic wounds
Wound dressing

ABSTRACT

Wound dressing developed using bioactive materials has been a current area of research for treating chronic non-healing wounds owing to its high demand. Here, we report the fabrication and evaluation of nanofibrous matrix based wound dressings using biopolymer poly(vinyl alcohol) (PVA) incorporated with silk sericin (SS). SS extracted from the cocoons of mulberry variety *Bombyx mori* and non-mulberry variety *Antheraea assama* has been used to develop two types of blended mats. Herein, SS based nanofibrous dressings fabricated using electrospinning technique were thoroughly characterized and evaluated for wound healing applications. The developed SS based nanofibrous mats ranged from 130 to 160 nm in diameter with micro to nanoporous structure. The dressings were endowed with free radical scavenging capacity, antibacterial activity, swelling capacity, and biocompatibility due to incorporation of SS. Furthermore, murine fibroblasts (L929) and human keratinocytes (HaCaT) cultured on the PVA-SS blended mats showed higher proliferation as compared to pristine PVA mats as observed over a period of 14 days ($p \leq 0.01$). The blended mats also showed spread out morphology of cells in comparison to spherical clumps formed on PVA mats. In addition, SS from both silk types exhibited excellent antioxidant potential without hampering the cell viability even under H_2O_2 driven oxidative stress. Moreover, SS (both types) released from the nanofibrous mats also healed the wounds at thrice the rate of control under *in vitro* conditions. Furthermore, subcutaneous implantation of nanofibrous mats in mice showed *in vivo* tolerance of the blended nanofibrous mats observed over four weeks without eliciting any inflammatory reactions to the host tissue. Taken together, the developed silk sericin-based dressings signify an attractive substrate for treatment of chronic wounds like diabetic foot ulcers.

1. Introduction

Skin is the largest organ of our body which covers all other organs and serves as a protective barrier against the environment. It has a high rate of repair mechanism leading to timely and orderly repair, but sometimes the pathological conditions like diabetes, obesity, and other diseases render a loss in its renewal capacity [1,2]. A failure in the wound healing process also occurs in case of large and deep wounds which exceed the patient's self-ability to heal. In addition, loss of the integrity of large skin portion or chronic cutaneous wounds may lead to major disabilities or fatal consequences. More than 300,000 deaths have been estimated by the World Health Organization (WHO) due to burn injuries and approximately 6.5 million people are suffering from chronic skin ulcers caused by diabetes mellitus, prolonged pressure, or venous sores [3].

Wound healing is a dynamic and interactive process which begins with hemostasis and includes three sequential phases: inflammation, tissue formation or proliferation, and tissue remodeling [4]. Obstruction in the healing pathways often leads to wound chronicity and thereby delays the healing rate from days to months. The chronic inflammatory stage at the very beginning of healing process is often a major cause of chronic cutaneous ulcers like diabetic foot ulcers (DFU) [5]. Prolonged inflammation at the site of injury halts fibroblast migration and prevents extra cellular matrix (ECM) formation, ultimately obstructing the healing process [5]. Chronic inflammation is also associated with inducing oxidative stress to the wound site and influencing the healthy cells residing in the wound edge to become senescent and stops migration [5]. Thus, inflammation phase of healing process should not be prolonged and therapeutic treatments preventing the persistent inflammation should be applied to treat chronic cutaneous wounds.

Currently, the commercially available wound dressings mostly focus

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https://doi.org/10.1016/j.msec.2018.04.077
Received 13 September 2017; Received in revised form 6 March 2018; Accepted 25 April 2018
Available online 27 April 2018
0928-4931/

on achieving antibacterial activity or covering the wounds to prevent infection of wounds [6]. Negative pressure therapy, silver dressings and implantation of artificial grafts are the common strategies being applied in the hospitals at present [7,8]. However, lack of bioactivity in the available commercial dressings has brought a huge gap in the availability and demand. The choice of biomaterial for the fabrication of wound dressings plays a crucial role in bridging this gap in recent years. There is a huge demand of natural biomaterials with inherent bioactive properties like antioxidant behavior, antimicrobial potential, cell binding, and ECM regulating properties. The unmet clinical challenges still exist to cure chronic skin wounds and thus there is a great demand of effective wound dressing. In this line, development of bioactive wound dressings has become a prime focus of scientists working on wound healing applications. Functionalization of biomaterials or biopolymers by incorporating bioactive molecules like growth factors, antioxidant molecules, and antimicrobial compounds are the common strategies by which accelerated wound healing has been achieved till date [9–12]. The present study deals with the development of wound dressings made up of silk sericin (SS), a natural protein biomaterial which itself is a bioactive compound and thus does not require additional functionalization of the substrate. SS possesses beneficial properties like biocompatibility, biodegradability, moisture retention ability, antioxidant and antibacterial activities [13–16]. SS is a glycoprotein contributing to almost 30% mass of silk cocoons which usually gets discarded as a waste product from the textile industries [13]. SS has also been explored as a serum substitute owing to its bioactivity on enhancing cell adhesion and promoting cell proliferation [17]. Significant research on the SS protein in last decade has attested its potential for tissue regeneration and medical applications. Therefore, SS previously considered as an industrial waste product can be utilized in a productive way opening new portals of medical therapeutics.

Tissue regeneration potential of SS from mulberry silkworm *Bombyx mori* has been investigated by conjugating the SS with other biomaterials like collagen, gelatin, chitosan, poly(vinyl alcohol) (PVA), poly (ι-lactide-*co*-ε-caprolactone) (PLCL) in various formats like sponge, nanofibrous mats, hydrogels and films [18–22]. Recently, SS from non-mulberry silkworms like *Antheraea assama* and *Philosamia ricini* has gained much attention in the biomedical and tissue regeneration applications owing to its bioactivity [14,20]. However, their application as wound dressing substrates is not much explored. The present study deals with the development of nanofibrous mats using SS from non-mulberry silkworm *Antheraea assama* which is endemic to India and possess additional properties that are beneficial for wound dressing applications [13]. The hydrophilic nature of SS requires a supportive substrate for a stable structure [13]. Therefore, it has been blended with a stable and inexpensive biomaterial PVA which has been widely explored for the development of wound dressings [9,23]. PVA was selected as a base material due to its properties like high swelling capacity, high elasticity, biocompatibility, water-solubility, durability, and low cost [23,24]. Moreover, it is an FDA approved polymer and it also provides ideal viscosity for electrospinning [9]. Nanofibrous mats have been developed using electrospinning technique as it is the best-suited format for wound dressings owing to the biomimetic structure, moisture retention properties and scalability [25,26]. The porous nature of electrospun mats provides suitable semi-occlusive properties by absorbing the wound exudates and allowing appropriate permeation of nutrients and gases, thus aiding in faster wound healing process [9]. Our recent study proved the antioxidant potential of SS from both mulberry and non-mulberry silkworm variety [14]. Thus, the antioxidant activity of SS may help in scavenging the reactive oxygen species (ROS) generated by senescent cells during the chronic inflammation. SS-based wound dressings may also easily prevent the prolonged inflammation and switch the wound healing process to proliferatory phase aiding in the accelerated healing of chronic wounds.

The present study focuses on the facile fabrication of SS-based wound dressings and assessment of their physical and biological properties ideal for wound healing applications. Biocompatibility of the nanofibrous mats has been investigated under both *in vitro* and *in vivo* studies by examining cytokine secretion from immune cells and subcutaneous implantation in mice, respectively. We have also delved into the substrate's ability to scavenge free radicals, improve cell viability under H_2O_2 driven oxidative stress, antimicrobial activity, and wound healing attributes under *in vitro* conditions. Our current study thus demonstrates the development of a functional wound dressing for treating chronic wounds that get stuck in the persistent inflammation phase thereby aiding in accelerated wound healing.

2. Experimental section

2.1. Extraction of silk sericin (SS) protein

Cocoons of *B. mori* and *A. assama* were obtained from local sericulture farm, Guwahati, Assam, India. For the extraction of silk sericin protein, the alkaline degumming method was performed [13,16]. Briefly, the cocoons were cut into smaller pieces, washed with water, and boiled in 0.02 M Na_2CO_3 solution (3 g L^{-1}) for 30 min (Himedia, India). The degummed silk fibers were removed and sericin solution was further boiled to concentrate the SS. The obtained solution was centrifuged at 8000 rpm for 15 min. The supernatant containing SS was filtered out prior to dialyzing it in Mili-Q water for 48 h using a 12 kDa dialysis membrane (Sigma Aldrich, USA). The obtained aqueous SS solution was subsequently freeze dried to obtain sericin powder. Sericin obtained from *A. assama* cocoons was termed as *A. assama* silk sericin (AASS) and that from *B. mori* cocoons was termed as *B. mori* silk sericin (BMSS).

2.2. Fabrication of sericin-based nanofibrous mats

PVA (LobaChemie Pvt. Ltd., India) polymer was used as the base material to fabricate the nanofibrous mats. The PVA granules and dried sericin powder were dissolved in lukewarm water in the ratio of 8:1 (w/w) and mixed thoroughly using a magnetic stirrer till a homogenous mixture was obtained. This solution was used for the fabrication of nanofibrous mat using electrospinning machine (E-spin nanotech, India). Briefly, the PVA-SS solution was filled in a syringe and spilled with a flow rate of 1 mL/h *via* a 21 gauge blunt needle. Rotating drum collector (at a rotating speed of 300 rpm) covered with aluminium foil was placed at a distance of 10 cm to collect the nanofibrous mat. The high electric *v*oltage of about 12–15 kV was applied to make smooth and continuous nanofibers without beads. Pristine PVA mat was also fabricated as control by electrospinning the PVA (8% w/v) solution. PVA-SS blend nanofibrous mats developed from AASS and BMSS were named as P + AASS and P + BMSS respectively. The three different types of nanofibrous mats (PVA, P + AASS and P + BMSS) were developed using similar electrospinning parameters. The mats were then treated with 80% ethanol for inducing crystallinity in the nanofibers in order to provide integral stability.

2.3. Physico-chemical characterization of nanofibrous mats

Surface morphology and fiber diameter of the nanofibrous mats were analyzed using field emission scanning electron microscope (FESEM) (Zeiss, Model: Sigma). The samples were subjected to gold sputter coating prior to loading in the FESEM instrument. The diameter of nanofibers was determined from the FESEM images by measuring diameters of 50 randomly selected nanofibers from the magnified images using Image J software (Wayne Rasband, National Institute of Health, USA).

The infrared spectrum of the nanofibrous mats was recorded using Fourier transform infrared spectroscopy (FTIR) (Nicolet iS 10, equipped with a TE cooled DTGS detector). The spectra had a resolution of 4 cm^{-1} and were recorded in the range of 4000–500 cm^{-1}